

autosomes were selected from different sources, but mainly from the Marshfield Institute panel version 8a, were available for this genome scan. Markers map locations (in megabases (Mb)) were taken from the Human Genome NCBI resources (Built 31; <http://www.ncbi.nlm.nih.gov/genome/guide/human/>). The average intermarkers distance was 6.8 Mb ranging from 0 to 32 Mb. The highest and the lowest marker density was on chromosome 20 (3Mb) and 21 (12 Mb) respectively.

#### Neuromedin $\beta$ polymorphism (c.217 C>A or p.P73T) genotyping

The use of c.217 C>A or p.P73T nomenclature design the same polymorphism on the coding sequence (DNA or RNA) and the peptide sequence respectively and are interchangeable (Fig. 6). On the coding sequence a C at position 217 is translated by a P (proline; genetic code for a proline: CCC) at position 73 on the peptide sequence. Alternatively, an A at position 217 on the coding sequence is translated by a T (threonine; genetic code for a threonine: ACC) at position 73 on the peptide sequence. In the genetic code between parentheses, the letter in bold type and in *italic* design the mutated nucleotide of c.217 C>A. However, the c.217 C>A nomenclature will be mostly favored to facilitate the comprehension of this document.

#### PCR reaction

In a final volume of 6  $\mu$ l, 20 ng of genomic DNA were added to a mixture containing a final concentration of dNTP (Amersham Pharmacia Biotech Inc.), 30  $\mu$ M each; *Taq* DNA polymerase (QUIAGEN<sup>TM</sup>), 0.3 U; buffer 1X (10 X: TRIS-HCl, KCl,  $(\text{NH}_4)_2\text{SO}_4$  and 15 mM  $\text{MgCl}_2$ ; pH 8.7 (20°C)); flanking primers, 50 nM each. Following a 5-min denaturation step at 95°C, 30 PCR amplification cycles were performed as follows: denaturation at 95°C, 20 sec; annealing 57°C, 1 min; for 10 cycles and denaturation at 95°C, 20 sec; annealing at 52°C, 1 min; for the remaining 20 cycles. In the same well, the PCR mixture dNTP's were digested using Shrimp Alkaline Phosphatase (USB), 0.2 U (final volume: 11  $\mu$ l) for 15 min at 37°C followed by 20 min at 80°C. Mini-sequencing assay, based on research done by Sun et al (Sun, Ding et al. 2000, Nuc. Acids Res. 28:E68), was performed in a final volume of 16  $\mu$ l (in the same well); dTTP/ddNTP mix (dTTP, ddATP, ddCTP and ddGTP) (dNTP and ddNTP are

**AMENDED SHEET**